

Advances in Mössbauer Spectroscopy of Diiron-Oxo Proteins

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MÖSSBAUER EFFECT

or:

RECOILESS FREE NUCLEAR RESONANCE

- Resonance Absorption

$$\sigma_{\text{abs}} = \frac{\sigma_0}{2} \frac{\Gamma_\gamma}{\Gamma} = \frac{\sigma_0}{2} \frac{1}{(1+\alpha)}$$

σ_0 : Max. resonance cross section

Γ_γ : Width of γ -ray

$\Gamma = \Gamma_{\text{emission}} = \Gamma_{\text{absorption}}$: Total width

α : Coefficient of internal conversion
(Nuclear decay: IC vs. γ -ray emission)

- Ideal conditions for resonance:
 1. Emission and absorption lines have natural width
 2. Emission and absorption lines centered at same energy
- γ -ray experiments: 1 and 2 are violated

R. L. Mössbauer (1957)

- Resonance scattering of 129 KeV γ -ray from Ir¹⁹¹
(R = 0.05 eV, D = 0.1 eV at RT)
- At RT:

Emission and absorption overlap

Resonance observed

- To reduce resonance scattering:

Cooling of source and absorber

Instead: resonance scattering increased !!

Achieved 1 and 2 in single experiment

BASIC MÖSSBAUER PARAMETERS:

- Isomer shifts (δ_{Fe})

$$\delta_{Fe} = \frac{2\pi}{3} Ze^2 \{ |\Psi_s(O)|_{Absor}^2 - |\Psi_s(O)|_{Sour}^2 \} \{ \langle R^2 \rangle_e - \langle R^2 \rangle_g \}$$

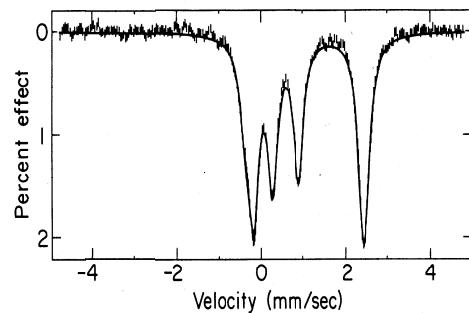
- Electric quadrupole splittings (ΔE_Q)

$$V_{ii} = \frac{2}{7} e(1-R) \langle r^{-3} \rangle \langle L_i^2 - 2 \rangle$$

$$\eta = \frac{V_{xx} - V_{yy}}{V_{zz}}$$

$$\mathcal{H}_{E_Q} = \frac{eQV_{zz}}{12} [3I_z^2 - I(I+1) + \eta(I_x^2 - I_y^2)]$$

$$\Delta E_Q = \frac{1}{2} e Q V_{zz} (1 + \eta^2/3)^{1/2}$$



DIIRON CENTERS IN BIOLOGY

- A class of metalloproteins: “Diiron-Oxo”
- Magneto-structural similarity:
(Anti)ferromagnetism
- Functional diversity
- Purple acid phosphatase (Uteroferrin): hydrolysis of phosphate esters
- Methane monooxygenase, ribonucleotide reductase: activation of O₂
- Hemerythrin (Hr): transport of O₂
$$\text{[Fe}^{2+}(\mu-\text{OH}^-)\text{Fe}^{2+}] + \text{O}_2 \rightleftharpoons \text{[Fe}^{3+}(\mu-\text{O}^{2-})\text{Fe}^{3+}\text{OOH}^-]$$

deoxyHr oxyHr

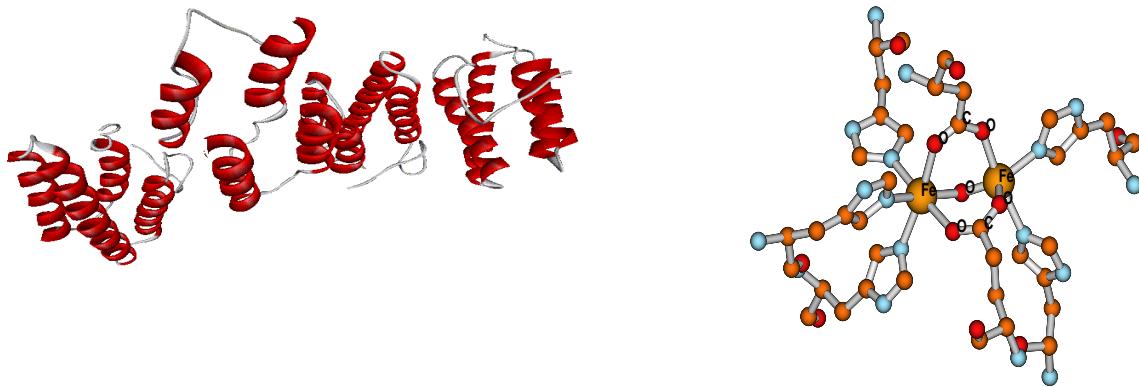


FIG. 1. **Left:** 2.0 Å structure of deoxyHr. **Right:** (μ -OH) $\text{bis}(\mu\text{-acetato})$ -bridged $\text{Fe}^{2+}(S_1 = 2)$ $\text{-Fe}^{2+}(S_2 = 2)$ center of deoxyHr [Stenkamp *et al.*].

HEISENBERG EXCHANGE INTERACTION

$$\mathcal{H}_{\text{ex}} = J \mathbf{S}_1 \bullet \mathbf{S}_2$$

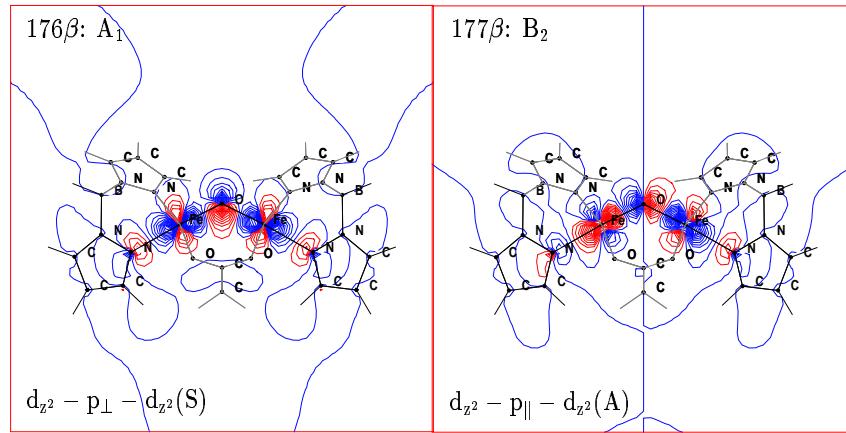
- Total spin operator, $\mathbf{S} = \mathbf{S}_1 + \mathbf{S}_2$, has eigenstates corresponding to $S_1 - S_2 \dots S_1 + S_2$

$$|S = \frac{3}{2}; M_S = \pm \frac{1}{2}, \pm \frac{3}{2} >$$

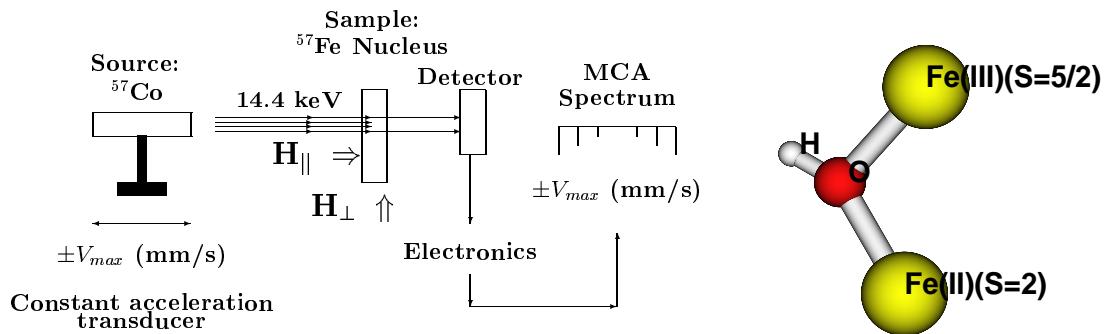
$$\frac{3}{2}J$$

$$|S = \frac{1}{2}; M_S = \pm \frac{1}{2} >$$

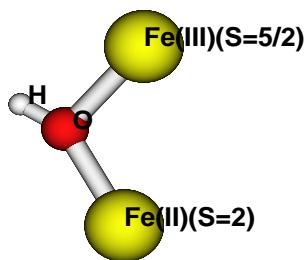
- Mechanism: superexchange



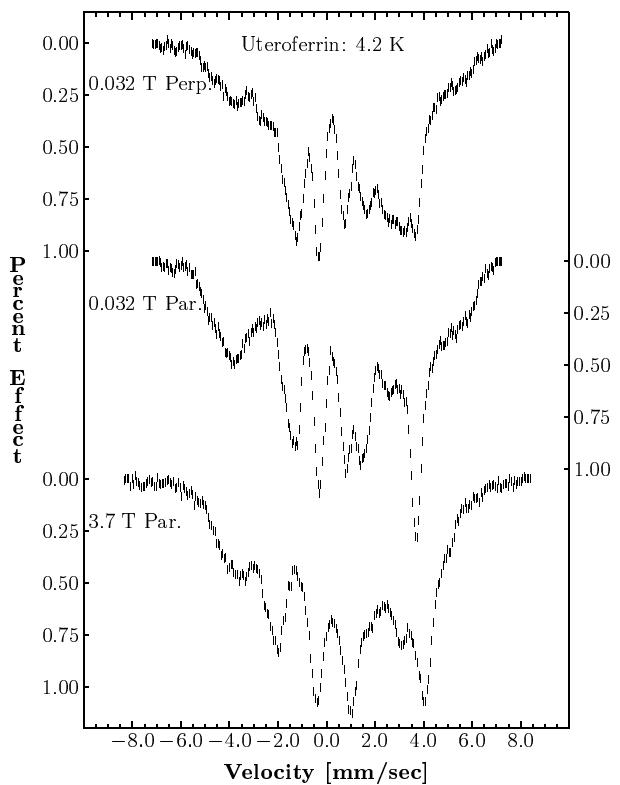
MAGNETIC MÖSSBAUER SPECTROSCOPY



- A powerful probe of diiron proteins
- ^{57}Fe spectra distinguishes non-equivalent irons
- Spectra are sensitive to:
 - Fe oxidation and spin states
 - Coordination and ligand fields
 - AF superexchange
 - Magnetic couplings
 - Hyperfine interactions
- Spectra:
 - Highly informative but complex



MÖSSBAUER DATA:



4.2 K EPR DATA:

		$S_1 - S_2$	\tilde{g}
Ufo (PAP)	$\text{Fe}^{3+} - \text{Fe}^{3+}$	$\frac{5}{2} - \frac{5}{2} = 0$	EPR Silent
Ufr (PAP)	$\text{Fe}^{3+} - \text{Fe}^{2+}$	$\frac{5}{2} - 2 = \frac{1}{2}$	1.94, 1.76, 1.56

Q: How to interpret data?

A: Need theoretical models:

LFT (Simulation)

DFT (Prediction)

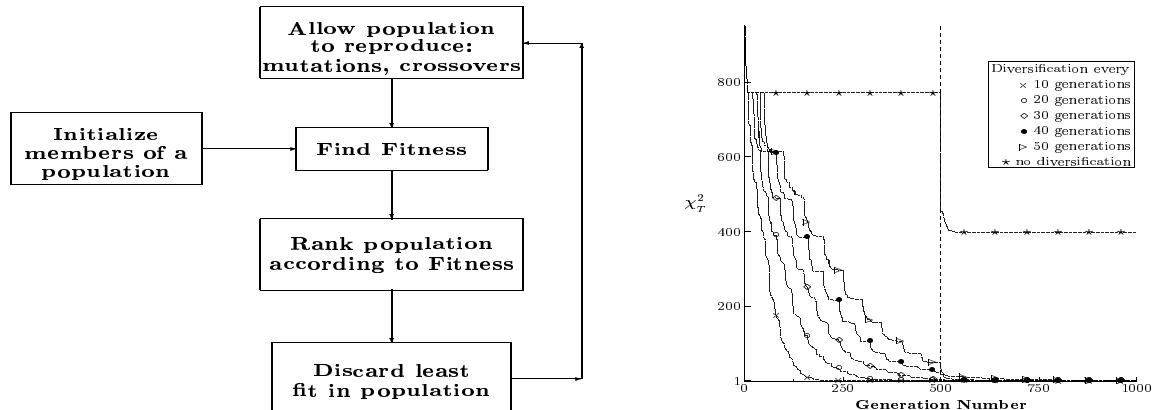
MODEL: SPIN HAMILTONIAN

$$\mathcal{H} = JS_1 \bullet S_2 + \sum_{i=1}^2 \{ S_i \bullet \tilde{D}_i \bullet S_i + \beta S_i \bullet \tilde{g}_i \bullet H + S_i \bullet \tilde{a}_i \bullet I_i + I_i \bullet \tilde{P}_i \bullet I_i - \beta_n g_n H \bullet I_i \}$$

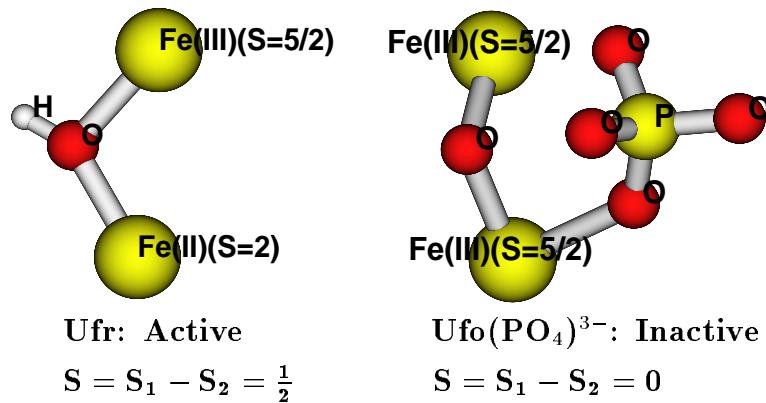
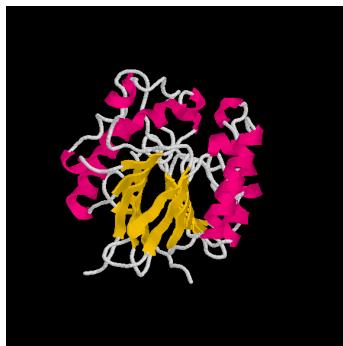
- Isotropic exchange interaction
- Zero field splittings, ZFS
- Electronic Zeeman interactions
- Magnetic hyperfine interactions
- Electric hyperfine interactions
- Nuclear Zeeman interactions

SEARCH IN MULTIPARAMETER SPACE:

Genetic Algorithms

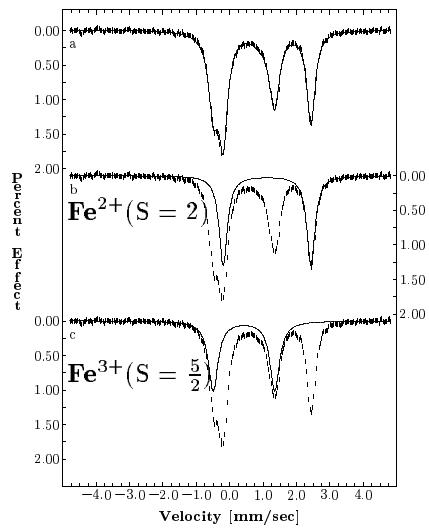


UTEROFERRIN (PAP)



- Pseudo-twofold symmetry, $\approx 35\text{kDa}$
- Ufr (pink): Activity at $\text{pH} = 4.9$, $\lambda_{\max} = 505\text{nm}$
- Ufo (purple): Inactive, $\lambda_{\max} = 550\text{nm}$

Ufr: $T = 120\text{ K}$, $H = 0\text{ T}$



		δ_{Fe} (mm/s)	ΔE_Q (mm/s)	S
UFr	Fe^{2+}	1.22	2.63	2
	Fe^{3+}	0.54	1.87	$\frac{5}{2}$
Ufo	Fe^{3+}	0.48	2.19	$\frac{5}{2}$
	Fe^{3+}	0.56	1.69	$\frac{5}{2}$

$$\xrightarrow{\Delta E_Q} |I_e = \frac{3}{2}; M_{I_e} = \pm \frac{3}{2} \rangle$$

$$\Delta E_Q$$

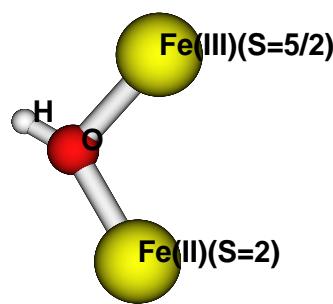
$$\xrightarrow{\Delta E_Q} |I_e = \frac{3}{2}; M_{I_e} = \pm \frac{1}{2} \rangle$$

$$\xrightarrow{\Delta E_Q} |I_g = \frac{1}{2}; M_{I_g} = \pm \frac{1}{2} \rangle$$

^aRodriguez, J. H.; Ok, H. N.; Xia, Y. M.; Debrunner, P. G.; Hinrichs *et al.* *J. Phys. Chem.*, **1996**, *100*, 6849.

SIMULATED MÖSSBAUER AND EPR DATA:

$$\mathcal{H} = JS_1 \cdot S_2 + \sum_{i=1}^2 \{ S_i \cdot \tilde{D}_i \cdot S_i + \beta S_i \cdot \tilde{g}_i \cdot H + S_i \cdot \tilde{a}_i \cdot I_i + I_i \cdot \tilde{P}_i \cdot I_i - \beta_n g_n H \cdot I_i \}$$

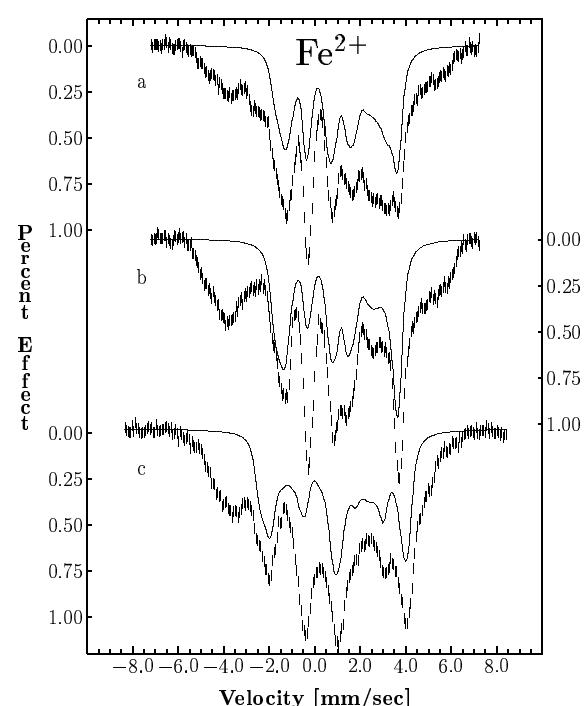
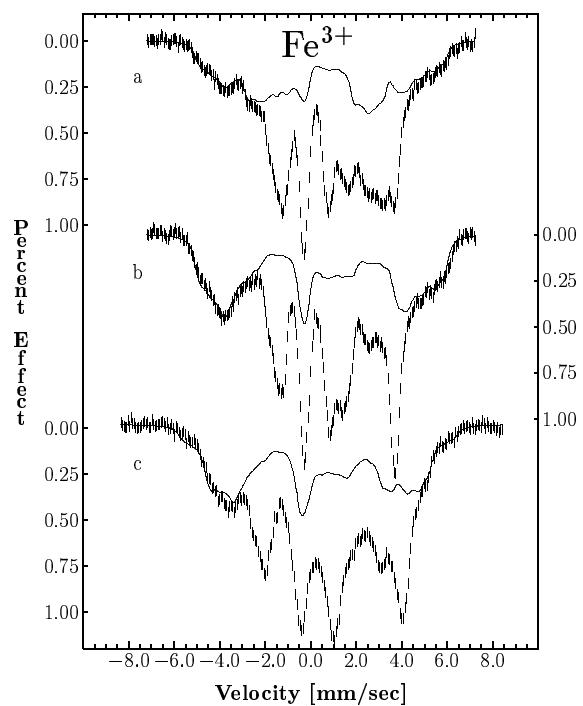
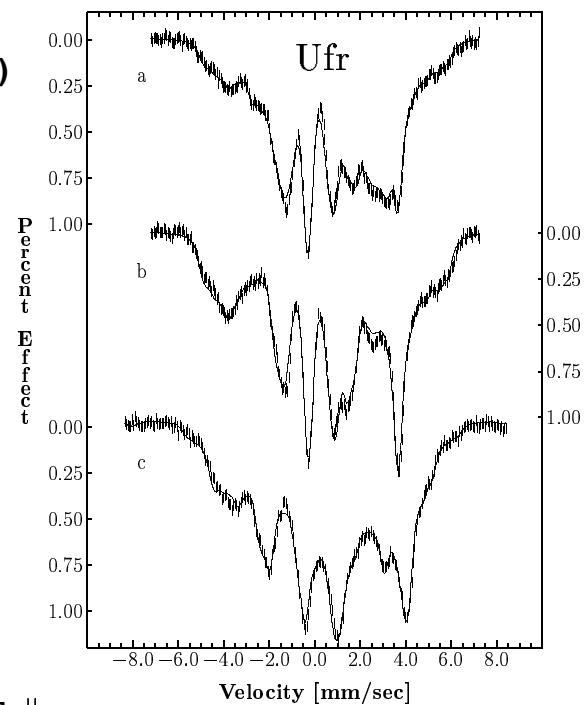


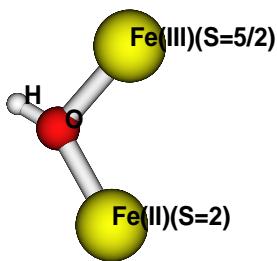
$$g_{\text{EPR}}^{\text{eff}} = (1.56, 1.73, 1.94)$$

$$g_{\text{Calc}}^{\text{eff}} = (1.56, 1.74, 1.95)$$

$T = 4.2 \text{ K}$

a) $0.032 \text{ T} \perp$, b) $0.032 \text{ T} \parallel$, c) $3.7 \text{ T} \parallel$





SPIN HAMILTONIAN OF Ufr:

$$\mathcal{H} = \mathbf{J}\mathbf{S}_1 \cdot \mathbf{S}_2 + \sum_{i=1}^2 \{ \mathbf{S}_i \cdot \tilde{\mathbf{D}}_i \cdot \mathbf{S}_i + \beta \mathbf{S}_i \cdot \tilde{\mathbf{g}}_i \cdot \mathbf{H} + \mathbf{S}_i \cdot \tilde{\mathbf{a}}_i \cdot \mathbf{I}_i + \mathbf{I}_i \cdot \tilde{\mathbf{P}}_i \cdot \mathbf{I}_i - \beta_n g_n \mathbf{H} \cdot \mathbf{I}_i \}$$

$J=34.66 \text{ cm}^{-1}$							
Site	S	D (cm ⁻¹)	E (cm ⁻¹)	$\tilde{a}/g_n\beta_n$ (Tesla)	$\mathcal{R}(\tilde{a}, \tilde{g}, \tilde{D} \rightarrow \tilde{P})$	\tilde{g}	ΔE_Q (mm/s)
Fe ²⁺	2	+10.81	+3.17	-(15.2,12.2,14.1)	(10,51,50) ^o	(2.11,2.21,1.99)	+2.74 0.40
Fe ³⁺	$\frac{5}{2}$	-0.10	0	-(21.3,21.2,17.8)	(29,67,65) ^o	(2.01,2.01,1.98)	-1.93 0.12

- Reproduces Mössbauer AND EPR data
- 1st determination of J from Mössbauer
- \mathcal{H}_{ZFS} strongly perturbs \mathcal{H}_{ex} : $\frac{D_2}{J} = 0.31$
- Anomalous hyperfine interactions

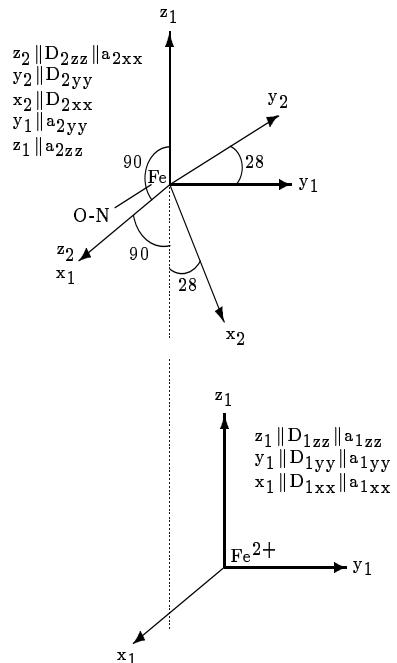
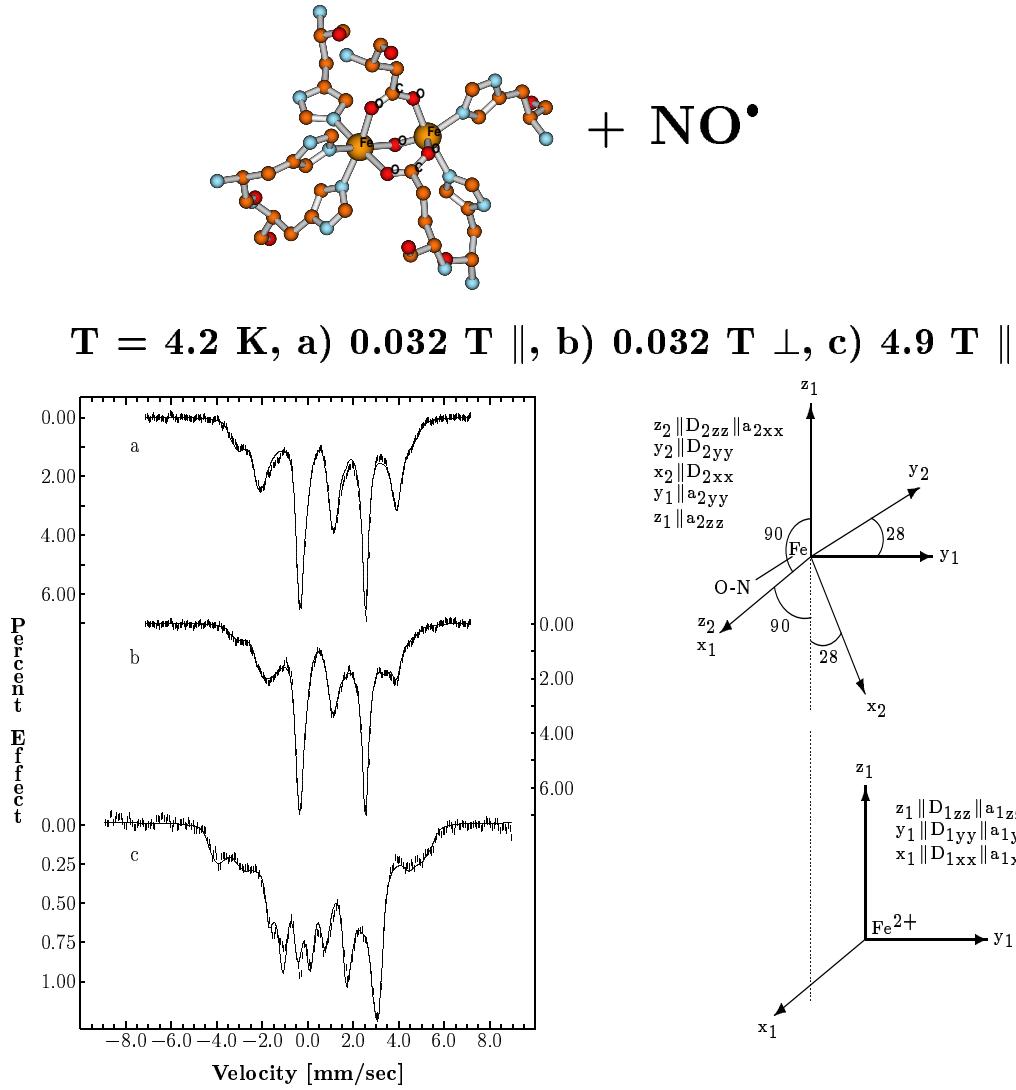
$$\mathcal{H}_{\text{Hf}} = \sum_{i=1}^2 \{ \mathbf{S}_i \bullet \tilde{\mathbf{a}}_i \bullet \mathbf{I}_i \}$$

$$a_{ii} = P[-\kappa + (g_{ii} - 2) + \frac{1}{14} < L_{ii}^2 - 2 >]$$

$$\Delta E_Q = \frac{1}{2} e Q V_{zz} (1 + \eta^2/3)^{1/2}$$

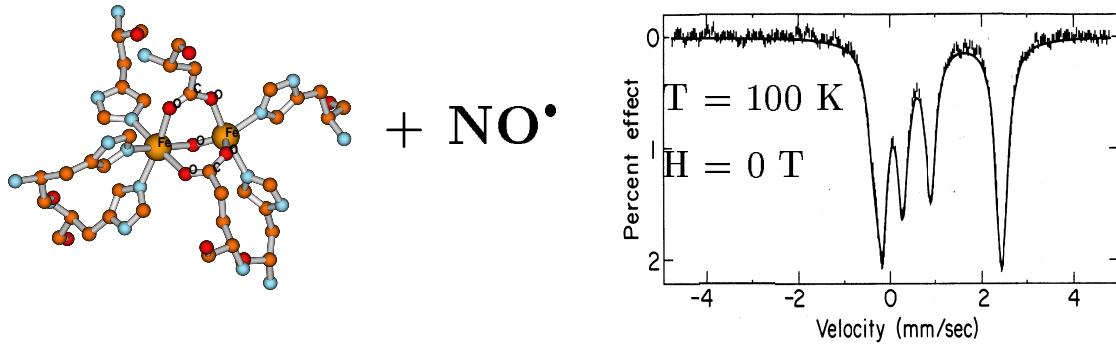
$$V_{ii} = \frac{2}{7} e (1 - R) < r^{-3} > < L_{ii}^2 - 2 >$$

DeoxyHrNO: SIMULATIONS OF SPECTRA



$J=27.83 \text{ cm}^{-1}$						
Site	S (cm^{-1})	D (cm^{-1})	E (cm^{-1})	$\tilde{a}/g_n\beta_n$ (Tesla)	$\mathcal{R}(\tilde{a} \rightarrow \tilde{D}, \tilde{g})$	\tilde{g}
Fe^{2+}	2	+5.96	+0.18	-(18.5, 10.4, 13.8)	$(0, 0, 0)^\circ$	(2.23, 2.24, 2.14)
$\{\text{FeNO}\}^7$	$\frac{3}{2}$	+18.93	+2.65	-(29.7, 25.0, ≈ 22)	$(0, 90, 28)^\circ$	(2.00, 2.00, 2.00)
0.00					$\tilde{g}_{\text{EPR}}^{\text{eff}} = (1.84, 1.84, 2.77)$	
					$\tilde{g}_{\text{Calc}}^{\text{eff}} = (1.83, 1.85, 2.78)$	

THE NEED FOR AB-INITIO: DFT



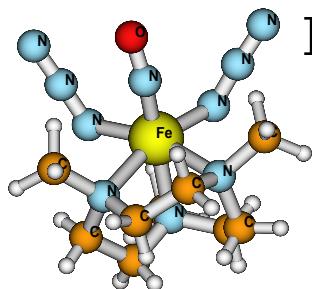
deoxyHrNO ^a	δ_{Fe} (mm/s)	ΔE_Q (mm/s)	S
Fe^{2+}	1.21	2.66	2
$\{\text{FeNO}\}^7$	0.68	0.61	$\frac{3}{2}$

- One site is $\text{Fe}^{2+}(S=2)$
- $|\Psi_s(0)|_{\text{Fe}^{3+}(S=\frac{5}{2})}^2 > |\Psi_s(0)|_{\{\text{FeNO}\}^7(S=\frac{3}{2})}^2 > |\Psi_s(0)|_{\text{Fe}^{2+}(S=2)}^2$

$\{\text{FeNO}\}^7$: Neither $\text{Fe}^{3+}(S=\frac{5}{2})$ nor $\text{Fe}^{2+}(S=2)$

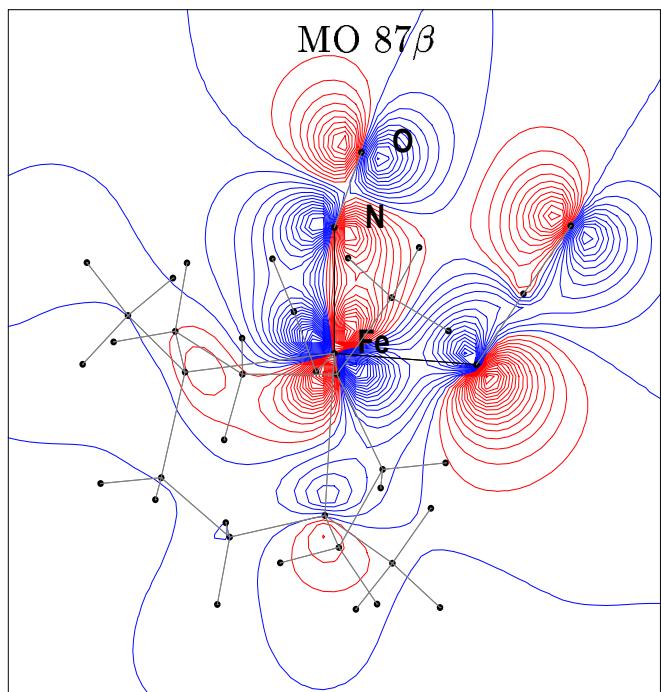
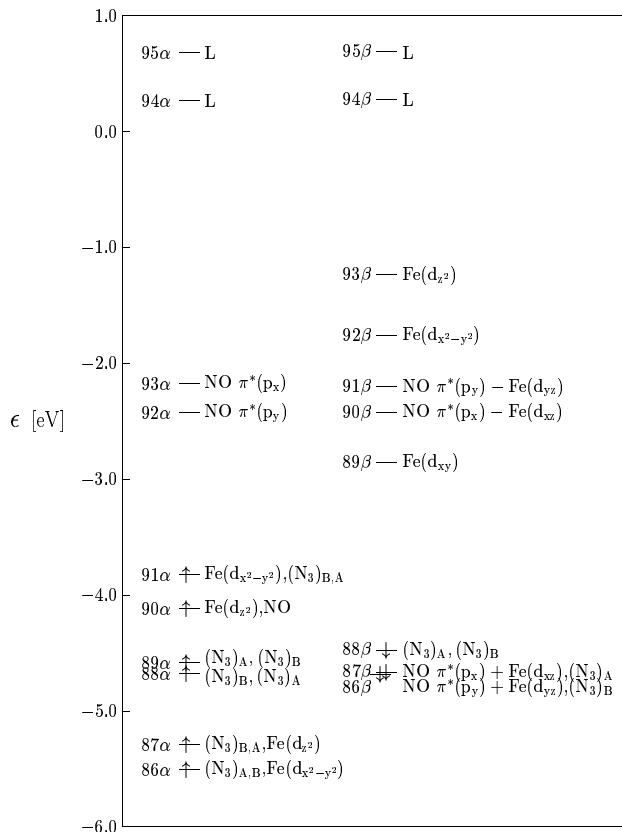
What is proper description of $\{\text{FeNO}\}^7$?

^aRodriguez, J. H.; Xia, Y. M.; Debrunner, P. G. *J. Am. Chem. Soc.*, **1999**, *121*, 7846.



KS-DFT^a OF $\text{Fe}(\text{NO})(\text{N}_3)_2\text{L}^{\text{b}}$

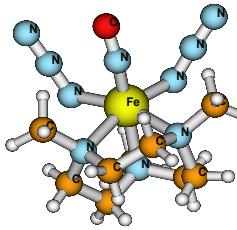
- **Experiment:**
 $\{\text{FeNO}\}^7 (\text{S} = \frac{3}{2}), \delta_{\text{Fe}} = 0.62 \text{ mm/s}$



- **Strong valence delocalization:**
Electrons shared by $\text{Fe}(\text{d}_{xz}, \text{d}_{yz})$ and $\text{NO}(\pi^*)$

^aRodriguez, J. H.; Xia, Y. M.; Debrunner, P. G. *J. Am. Chem. Soc.*, **1999**, *121*, 7846.

^b L = C₉H₂₁N₃. Pohl, K.; Wieghardt, et al. *J. Chem. Soc., Dalton Trans.*, **1987**, *Dalton Trans.*, 187.



KS-DFT^a OF $\text{Fe}(\text{NO})(\text{N}_3)_2\text{L}$

Ligand	Atom	Charge	Spin
	Fe	+1.358	+2.909
NO	N2	-0.095	-0.205
	O1	-0.158	-0.219
$(\text{N}_3)_A$	N5	-0.602	+0.044
	N11	+0.166	-0.005
	N12	-0.233	+0.121
$(\text{N}_3)_B$	N4	-0.616	+0.064
	N9	+0.172	-0.009
	N10	-0.231	+0.137

Atom	NAO	Occupancy	Spin
Fe	4s	0.302	+0.011
	3d _{xy}	1.170	+0.804
	3d _{xz}	1.415	+0.426
	3d _{yz}	1.401	+0.376
	3d _{x²-y²}	1.225	+0.720
	3d _{z²}	1.124	+0.569

- Fundamental differences between:
 $\{\text{FeNO}\}^7(S = \frac{3}{3})$, $\text{Fe}^{3+}(S = \frac{5}{3})$ and $\text{Fe}^{2+}(S = 2)$
- $\{\text{FeNO}\}^7$: Large occupancy of $3d_{xz}$ and $3d_{yz}$: Strong shielding on Fe(3s) electrons
 Reduced $|\psi_s(0)|_{^{57}\text{Fe}}^2$
 Large δ_{Fe} respect to $\text{Fe}^{3+}(S = \frac{5}{3})$

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